Carbonic anhydrase inhibitors based on sorafenib scaffold: Design, synthesis, crystallographic investigation and effects on primary breast cancer cells

Carbonic Anhydrase Inhibitors based on Sorafenib: Design, Synthesis, Crystallographic Investigation and Effects on Primary Breast Cancer Cells

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Abstract: Herein we report for the first time Carbonic Anhydrase Inhibitors (CAIs) of the sulfonamide, sulfamate and coumarin classes bearing the phenylureido tail from the clinically used drug Sorafenib, a multikinase inhibitor actually used for the management of hepatocellular carcinomas. All the final compounds were assayed on human (h) expressed CA isoforms I, II, VII and IX and showed quite interesting kinetic profiles. Among the sulfonamide ones, compounds **14-16** and **26** were selective in inhibiting the hCA IX with K_I values in the low nanomolar ranges (i.e. 0.7-30.2 nM). We explored the binding modes of such compounds by means of X-ray crystallographic studies on the hCA I in adduct with the sulfonamide **16** and a sulfamate **21**. Moreover, we antiproliferative properties of less investigated sulfamates **23** and **24** on breast tumor cell lines were investigated.

Keywords: Carbonic Anhydrase, Sorafenib, Carbonic Anhydrase Inhibitors, sulfonamide, sulfamate, coumarin

1.Introduction

The 15 human (h) expressed Carbonic Anhydrases (CAs, EC 4.2.1.1) are zinc (II) dependent metalloenzymes, differently distributed at subcellular and tissue level [1,2]. hCAs are involved in vital physiological reactions by means of the reversible conversion of carbon dioxide to bicarbonate and protons [3]. Abnormalities in hCA expressions usually result in diseases, such as hypertension, glaucoma, epilepsy, obesity and altitude sickness and cancers [3,4]. Carbonic anhydrase inhibitors (CAIs) of the sulfonamide type or their bioisosteres, are routinely used as drugs. The most used are Acetazolamide (AAZ), methazolamide (MTZ), dichlorophenamide (DCP), brinzolamide (BRZ), Sulthiame (SLT), Zonisamide (ZNS), the sulfamate Topiramate (TPM) and various COXIBs with dual CA-cycloxygenase activity such as Celecoxib and Polmacoxib [3,5]. Besides the sulfonamide moiety, many new types of CAIs acting as zinc binders were validated (i.e. dithiocarbamates [6–9] xanthates [10] and monothiocarbamates [11]). In addition new moieties include molecules able to interact to the zinc coordinated water/hydroxide ion (i.e. phenols [12–16], carboxylic acids [16,17], polyamines [18,19], thiooxycoumarins [20,21] and the sulfamic acid group from hydrolysis of sulfocoumarins [22–28]), compounds occluding the entrance of the CA active site (cinnamic acid derivatives from *in situ* hydrolyzed coumarins [20,29–32]) and out of the site CAIs such as the 2-(benzylsulfonyl)-benzoic acid [33]. Although many CAI chemical moieties were reported, the selectivity in targeting specific enzymatic isoforms was not granted. Therefore, any medicinal chemistry application of CAIs resulted hampered. Interestingly, the decoration of the CAI warhead by means of appropriate tails revealed to be a valid strategy to overcome the lack of enzymatic selectivity. In particular the arylureido-tails were particularly efficient in solving the selectivity issue among the hCA isoforms [34], and in particular such a moiety revealed preferential inhibition towards the tumor associated isoforms (i.e. hCAs IX and XII) over the cytosolic off-target ones (i.e. hCAs I and II) *in vitro*. Such results were confirmed in *in vivo* experiments using an appropriate breast cancer model [32, 33], and it was rationalized at molecular level by using X-ray crystallography techniques. The flexibility of the ureido-group is claimed responsible in allowing the entire molecule to better fit within the CA active site, and thus in maximizing the interactions occurring between the ligand and the amino acid residues exposed at the enzymatic cleft [35, 37]. The compound (4-(3-(4-fluorophenyl)ureido)benzenesulfonamide, also known as **SLC-0111,** was obtained by application of such a strategy and currently is facing phase II clinical trials in combination with gemcitabine for the treatment of metastatic pancreatic ductal cancer [38]. **SLC-0111** is selective inhibitor of the hCA IX isoform which has almost exclusive expression in hypoxic tumors [39–41], and is mainly responsible of the extracellular pH modulation [36, 39–41]. A recent application of the ureido tail strategy was reported by our group in the literature with ureido- substituted sulfamates, coumarins, 4-hydroxy-3-

(3-(phenylureido)benzenesulfonamides, *N*'-phenyl-*N*-hydroxyureas and *N*-aryl-*N*'-ureido-*O*-sulfamates [20,42–46].

Our pursuit to find more efficient ureido-bearing molecules to treat hypoxic tumors directed us to Sorafenib (Nexavar®), an orally available multikinase inhibitor approved by FDA to treat hepatocellular carcinoma (HCC). Clinical studies showed Sorafenib to exert promising effects on thyroid cancer, myeloid leukemia, mesothelioma and prostate cancers [47]. HCC is one of the most common cancers associated to high ratio of mortality [48,49,47]. Sorafenib was proved to extend a median time of progression in patients with advanced HCC and life span of patients with an average of three months [50, 52].

2. Results and Discussion

2.1. Drug Design and Chemistry.

In light of our discoveries on arylureido containing molecules for the management of hypoxic cancers, we decided to merge the structures of Sorafenib and **SLC-0111** to obtain a novel series of CAIs potentially useful to manage such types of pathologies, as schematically represented in **Figure 1**.

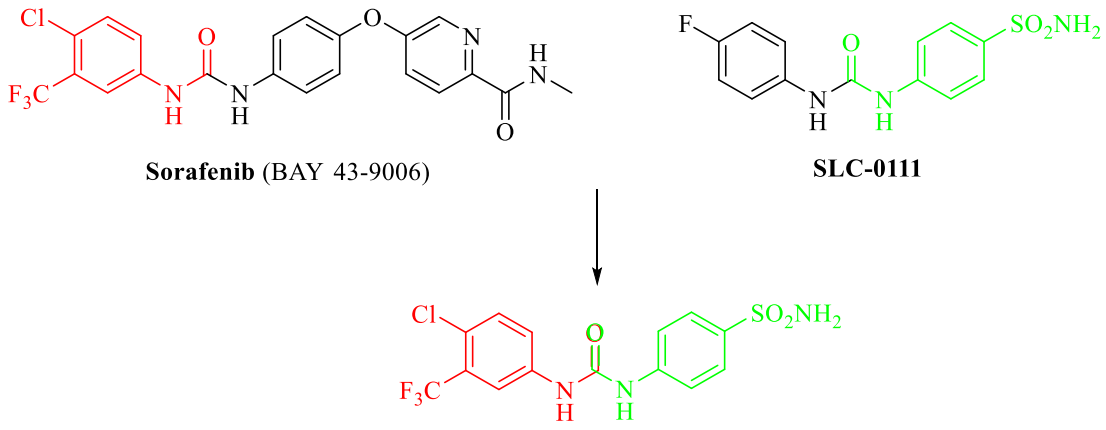
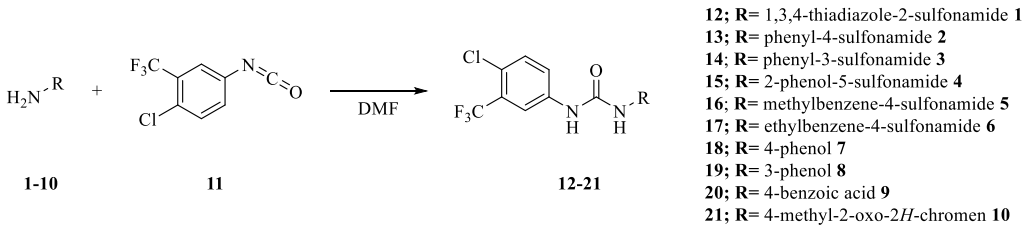


Figure 1. Sorafenib, **SLC-0111** and **13** obtained by our synthetic strategy.

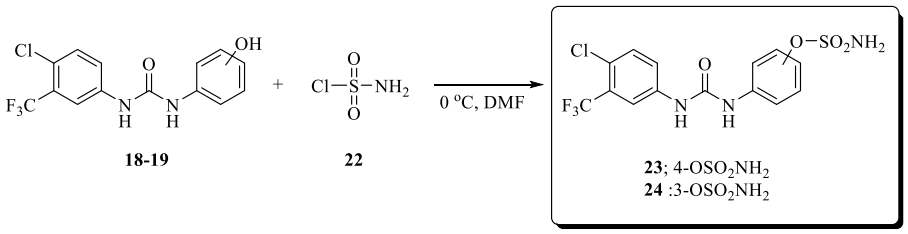
Our design strategy was further extended to include series of compounds bearing the sulfonamides, the sulfamates and the coumarin CAIs warhead.

As reported in **Schemes 1-3**, final compounds **12-17** and **21** were easily obtained by coupling the amino containing materials **1-6** and **10** with the commercially available 4-chloro-3-(trifluoromethyl)phenyl isocyanate **11**. The same reaction was applied to the synthesis of intermediates **18-20.** In particular **18** and **19** were further decorated with the sulfonamide bioisosteres

sulfamate moiety to afford **23** and **24** (scheme 2), whereas the latter **20** was activated by means of its NHS ester **25** which was used to obtain elongated CAIs of the sulfonamide (i.e. **26, 27, 29, 31**) [51] and of the coumarin type (i.e. **33** and **35**) [32].



Scheme 1. General synthesis of ureido derivatives **12-21**



Scheme 2. General synthesis of sulfamates **23-24**

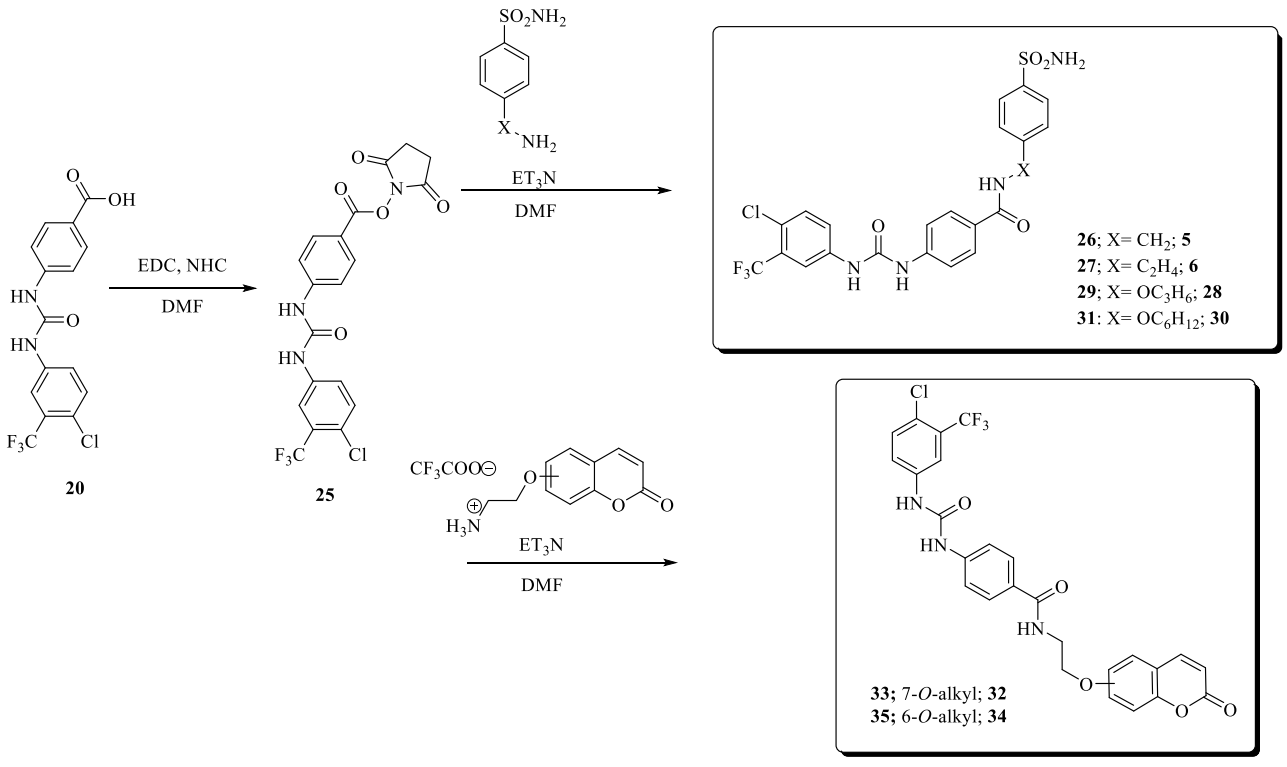


Figure 3. General synthesis of carbamides **26-27, 29, 31, 33, 35**:

All compounds were characterized by means of 1H-, 13C-, 19F-NMR and ESI mass spectroscopy techniques.

2.2. Carbonic Anhydrase Inhibition

The reported compounds having the sulfonamides (i.e. **12-17**, **26, 27**, **29**, **31)**, the sulfamates (i.e. **23, 24**) and the coumarin (i.e. **21, 33**, **35**) moieties were tested in vitro for their inhibitory properties against the physiologically relevant hCA isoforms hCA I, II, VII, IX in comparison with **Sorafenib** and the clinically used drug Acetazolamide (**AAZ**) by means of the stopped-flow carbon dioxide assay [52] (Table 1).

	K_I (nM)*			
Cmp	hCA I	hCA II	hCA VII	hCA IX
12	0.63	0.24	0.38	0.13
13	84.5	1.8	198.5	3.9
14	6422.9	71.5	916.3	1.8
15	4042.3	744.5	893.0	30.2
16	513.6	51.4	801.2	0.7
17	913.4	385.6	911.3	3972
21	>10000	>10000	>10000	1588.1
23	48.7	3.7	5.7	1.3
24	39.3	4.0	3.5	0.8
26	432.1	90.0	210.1	4.2
27	4545.1	2660.2	488.1	3298
29	4601.0	932.9	363.3	188.3
31	6348.3	5761.4	584.9	102.4
33	>10000	>10000	>10000	3412.4
35	>10000	>10000	>10000	2254.4
Sorafenib	>10000	>10000	8136.4	>10000
SLC-0111**	5080	960	8550	45.1
AAZ	250.0	12.1	6.0	25.8

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values). ** From reference [35, 53]

The inhibition data are showed in Table 1 and the following SARs are below discussed:

(i) The off target cytosolic isoform hCA I was inhibited by all compounds with zinc binding properties **12-17**, **23-24**, **26-27**, **29**, **31** with a variety of potencies. The derivative of acetazolamide **12** acted as sub-nanomolar hCA I inhibitor with a K_I of 0.63 nM. Sulfanilamide derivative **13** and two sulfamates **23-24** were potent hCA I, possessing inhibition constants ranging between 39.3 to 84.5 nM. Remaining compounds were medium potency hCA I inhibitors, they act as more potent inhibitors compare to acetazolamide **AAZ** (K_I of 250 nM). Remaining sulfonamides showed a lower affinity for hCA I, with K_Is in the range of 432.1-6422.9 nM. The coumarins **21, 33**, **35** did not inhibit the slow cytosolic isoform (hCA I) with inhibition constants > 10 μM. This result has been observed before by many coumarin derivatives [20,29–31].

(ii) The second abundant cytosolic isoform hCA II was inhibited by sulfonamides and sulfamates with K_Is in a range of 0.24-5761.4 nM. Compounds **12-13** and **23-24** acted as potent hCA II inhibitors with K_Is of 0.24-4.0 nM. **AAZ** derivative **12** was the most potent inhibitor of cytosolic isoform hCA II with a K_I of 0.24 nM, the second most potent inhibitor was sulfanilamide derivative **13** with a K_I of 1.8 nM. Two sulfamates **23-24** also acted as potent inhibitor of this isoform with K_Is of 3.7 and 4.0, respectively. Remaining sulfonamide derivatives **14-17** were from medium to less potent inhibitors of this hCA isoform with variety of K_Is between 51.4-744.5 nM. We observed a dramatic change of K_I (39.7 fold) by switching sulfonamide group from para- position **13** to meta- position **14** which had an inhibition constant of 71.5 nM. When a hydroxy group was appended at the para- position towards the sulfonamide of **14** to afford **15**, the inhibition constant (K_I) lowered to 744.5 nM (10.4 fold). Very recently we investigated inhibition properties of ureido- derivatives of **4** which are same chemotype of **15** which were also weak inhibitors of hCA II isoform [44]. The introduction of aliphatic linker between the ring bearing zinc binding group (ZBG) and urea group (**16-17**) was showed to lower hCA II inhibitory properties, with K_Is 51.4 for **16** and 385.6 for **17** (28.6, 214.2 fold, respectively compared to **13**). When a benzamide group was inserted in between the phenylureido tail and inhibitor heads to investigate inhibition properties of longer compounds, **26-27**, **29**, **31**. These compounds were weak inhibitors of hCA II isoform with K_Is in range of 90-5761 nM. We previously obtained the X-Ray crystal structures of ether tailed sulfonamides whose tails were located at the outer rim of active site of hCA II [51]. It is reasonable to hypothesize that compounds **29, 31** have similar behavior. The coumarins 2**1, 33**, **35** did not inhibit the slow cytosolic isoform (hCA I) with inhibition constants > 10 μM. This result has been observed before by many coumarin derivatives.

(iii) Compounds investigated here also showed a variety of different inhibition constants on cytosolic isoform hCA VII. The sulfonamides and the sulfamates were inhibited the hCA VII with K_Is in the range of 0.38-916.3 nM. Again, acetazolamide derivative **12** was the most potent inhibitor with a K_I of 0.38 nM. Followed by two potent sulfamates **23-24** with K_Is 5.7, 3.5 nM, respectively. These three compounds were acted as more effective inhibitors compare to **AAZ** (K_I of 6 nM). Remaining compounds were weak inhibitors of hCA VII isoform K_Is in the range of 198.5-916.3 nM. The coumarins did not inhibit cytosolic isoform VII as well with K_Is > 10 μM.

(iv) Sulfonamides and sulfamates reported here were effective as medium potency inhibitors of tumor associated extracellular isoform hCA IX with K_Is ranging 0.13-3972 nM. **12**-**16**, **23**-**24**, **26** showed excellent CA IX inhibitory profiles with K_Is in the range of 0.13-30 nM. **29** and **31** were weak inhibitors of this isoform with K_Is of 188.3 and 102.4 nM, respectively. Two sulfonamide derivatives **17** and **27** were ineffective inhibitors with K_Is of 3972 and 3298 nM, respectively. Coumarin derivatives **21**, **33** and **35** were also poorly inhibited tumor associated isoform hCA IX (K_Is of 3972 to 3412.4 nM). **AAZ** derivative **12** was again a sub-nanomolar, most potent inhibitor of the series with a K_I of 0.13 nM and again more potent inhibitor compare to **AAZ** (K_I of 25.8 nM). Followed by **16** which is also a sub-nanomolar inhibitor of this isoform (K_I of 0.7 nM). Insertion of an ethyl- group instead of methyl- between the ureido linker and sulfonamide possessing phenyl ring dramatically decreased inhibitory profile, **17** showed ineffective inhibitory profile (5674.29-fold). Whereas, deletion of the methyl group results **13 (**the derivative of **SLC-0111)** in 5.6-fold weaker inhibitory of this isoform (K_I of 3.9 nM). Manipulation of the sulfonamide group from 4-position to 3-position has enhanced the inhibitory profile 2.16-folds **14** (K_I of 1.8 nM). Whereas an insertion of -OH group at para position of Sulfonamide at **14** gives **15** that showed dramatic decrease of inhibition of this isoform 16.78-fold (30.2 nM). Two sulfamates **23**-**24** were excellent inhibitor of this isoform with K_Is of 1.3 and 0.8 nM, respectively. This results were obtained previously ureido-possessing sulfamates [54]. To obtain longer inhibitor molecules a phenylcarbamate moiety was inserted between the ureido- tail and inhibitor heads to obtain **26**-**27**, **29** and **31**. The methylene benzenesulfonamide head possessing derivative **26** showed excellent inhibitory effect with a K_I of 4.2 nM whereas ethylbenzenesulfonamide derivative **27** was ineffective inhibitor with a K_I of 3298 nM, that shows a dramatic change by addition of methyl group. **29** and **31** were medium potency inhibitors with K_Is of 188.3 and 102.4 nM, respectively. Coumarins **21, 33**, **35** inhibited transmembrane isoform with low efficiency (K_Is 1.59-4.31 μM), whereas did not inhibit any of cytosolic isoforms (CA I, II, VII), (Table 1).

Overall the kinetic data showed a preferential inhibition of the sulfonamide and sulfamate compounds on the hCA IX isoform with a

2.3. X-Ray crystallography.

We assessed the binding modes of compounds **17** and **23** within the active site of hCA I by means of X-ray crystallographic experiments. Our intention was to investigate a hCA isoform usually less considered since the majority of CA X-ray crystal adducts reported are related to the ubiquitous hCA II or to the tumor associated hCA IX mimic [16]. hCA I is abundant in red blood cells (at 1 μM concentration level), and it might influence the pharmacokinetics of drugs interacting with it or other CA isoforms of pharmacological interest.

The difference |Fo Fc| electron density maps showed well ordered structures within the active site of hCA I and clearly assignable to compounds **17** and **23** bounded by means of their sulfonamide and sulfamate group to the protein metal ion (**Figure 3**).

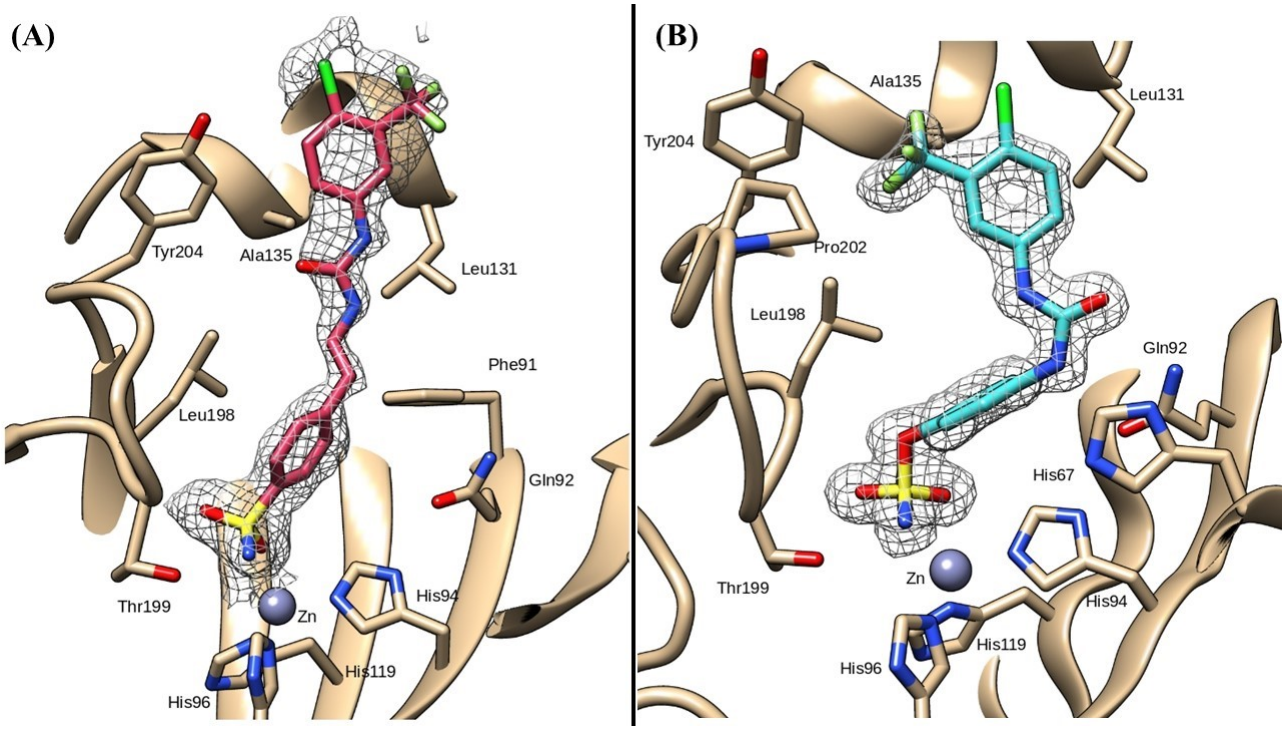


Figure 2. hCA I complexes of compound **17 (A;** PDB accession code 6I0L**)** and **23 (B;** PDB accession code 6I0J**)** at 1.4 and 1.35 Å resolution respectively**.**

A deep look into the structural features revealed **17** and **23** coordinated to the Zn (II) ion by means of the deprotonated sulfonamide and sulfamate moieties (SO_2NH^-, OSO_2NH^-), which in turn are also involved in a strong H-bond with the OH of Thr199 residue. One of the

sulfonamide/sulfamide oxygen is also involved in a hydrogen interaction with the amide nitrogen of Thr199. This coordination pattern, which is typical for all primary sulfonamides/sulfamates, is respected also in these cases [16]. The two inhibitors have a similar orientation inside the cavity, but **23** presents a bended conformation. This could be the result of the strong H-bond involvement of ureido-moiety of **23** with Gln92 residue further reinforced by a perpendicular π-stacking between the phenyl ring and the His94 at the bottom of the cavity site (**Figure 3**).

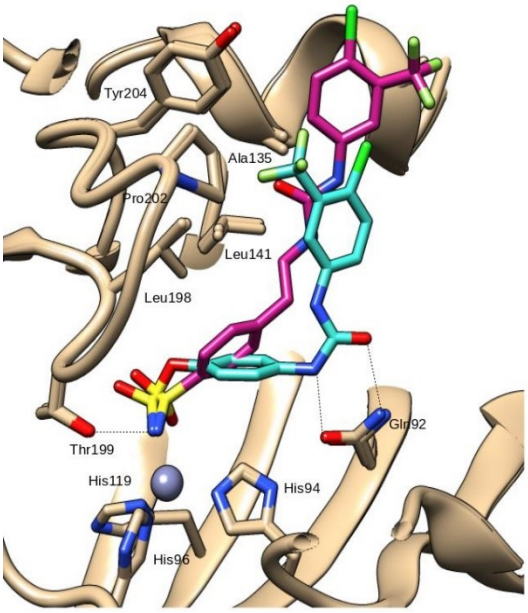


Figure 3. Superimposition of hCA I-**17** (purple) and **23** (cyan) adducts.

The 3-(trifluoromethyl)-4-chlorophenyl tail of **17** is involved in hydrophobic interactions with Ala135 and Tyr204 whereas the same tail moiety of **23** is slightly retained within the enzymatic cleft and makes hydrophobic interactions with Leu131 and Ala135.

The additional interactions of **23** when compared to **17**, within the catalytic cavity site (i.e. π stacking and the hydrogen bond interaction with Gln92) are reflected into the kinetic results on the same CA isoform. As reported in **Table 1** and above discussed, compound **17** is 18.8 fold less effective on hCA I when compared to **23** which is bound tighter.

2.4. Effects of Compounds 23 and 24 on Breast Tumor Cell Growth.

The in vitro obtained results led us to select less investigated sulfamate type inhibitors **23** and **24** (which are excellent inhibitors of hCA IX with K_Is between 0.8-1.3 nM, Table 1) for their effects on primary breast tumor HBL100, MDA.MB.231 and MCF7 cell lines in normoxic and hypoxic conditions.

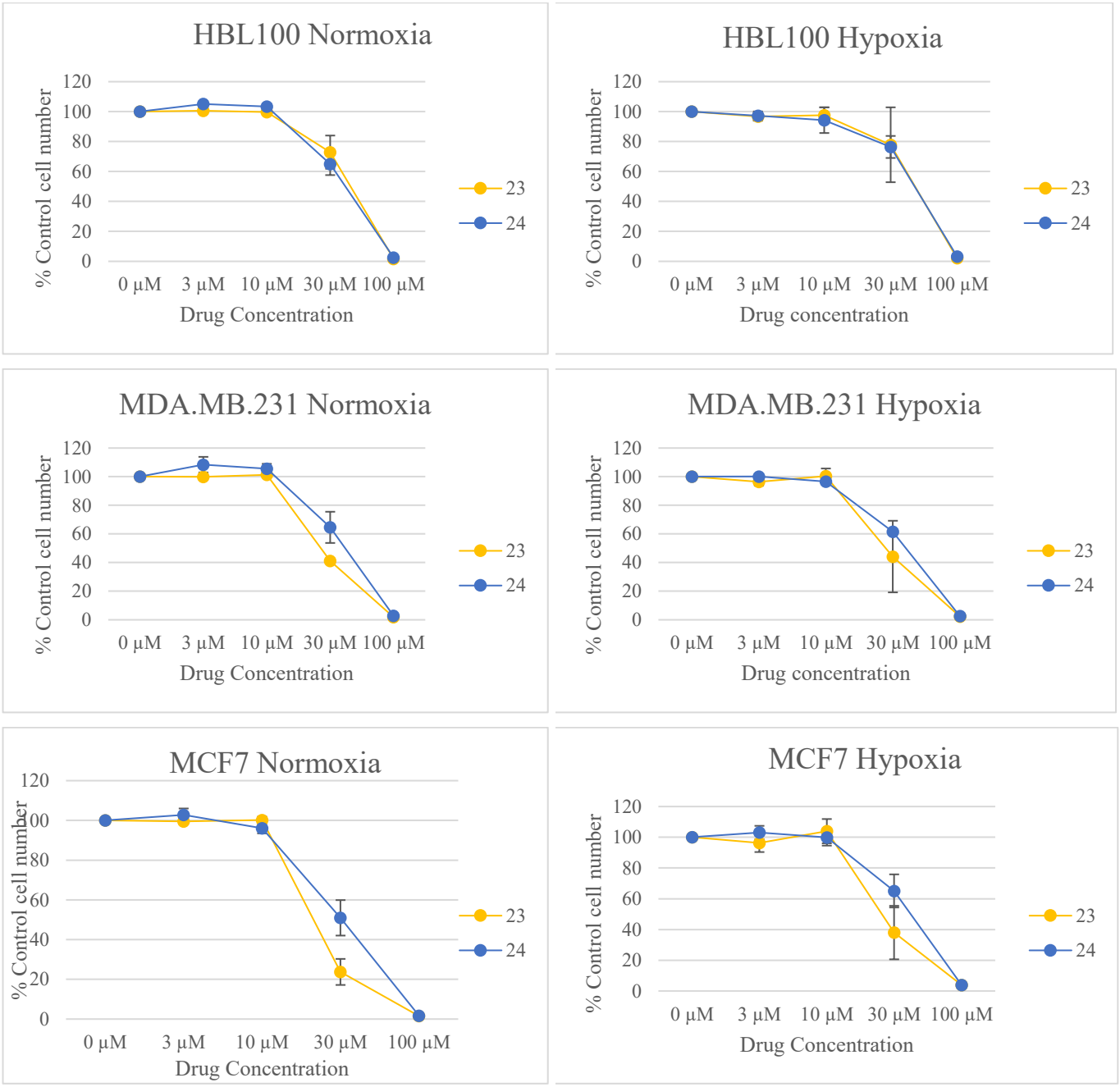


Figure 4. The effect of sulfamate type inhibitors 23 and 24 on proliferation of breast cancer cells in normoxic and hypoxic conditions.

HBL100, MDA.MB.231 and MCF7 breast cancer cells (1 x 10^3)/well, were allowed to adhere for 24h. Cells were either cultured in normoxia (21% O_2), or hypoxia (0.5% O_2), for a further 24 h before the addition of inhibitors at the concentrations indicated above and cultured in these conditions for a further 5 days. Proliferation was assessed using SRB assays. Results shown mean ± SEM (n=6; 6 replicates per experiment.

Figure 4 demonstrates that sulfamate type inhibitors **23** and **24** decreased the proliferation of all 3 breast cancer cell lines over the time period examined at concentration greater than 10 μM. At 30 μM, both sulfamates reduced proliferation by approximately 50%. This occurred regardless of the oxygenation status of the cells, with similar concentration responses to both inhibitors, in normoxic or hypoxic conditions and in all cell lines studied.

3. Conclusion.

Herein we reported a series of CAIs obtained by merging the multikinase inhibitor **Sorafenib** with the benzene sulfonamide hCA IX inhibitor **SLC-0111**. All compounds here share the same tail moiety of Sorafenib (i.e. 4-chloro-3-(trifluoromethyl)phenyl ureido group) and were assayed *in vitro* as inhibitors of four hCA isoforms of pharmacological relevance, the cytosolic isoforms hCA I, II and VII, and the tumor associated transmembrane isoform hCA IX. Interestingly the *in vitro* results showed a preferential inhibition of the tumor associated isoform with K_I values spanning between the low to medium nanomolar concentration. We determined the binding modes of compounds **17** and **23** on the hCA I isoform by means of X-ray crystallographic studies of their adducts with hCA I. Despite the canonical binding mode of the sulfonamide and sulfamate for the Zn (II) ion, the compounds showed different orientations within the cavity cleft. In particular the latter was involved in additional hydrogen a π stacking bondings which resulted in forcing the entire molecule to assume a curved conformation. The effects of such additional interactions were also evident from the kinetic viewpoint as **23** resulted 18.8 fold more potent in inhibiting the hCA I when compared to **17**.

Two sulfamates **23** and **24** were investigated for their antiproliferative activities on HBL100, MDA.MB.231 and MCF7 cell lines at normoxic and hypoxic conditions. Both sulfamates were found to decrease proliferation of these breast cancer cell lines at concentrations above 10 μM regardless of oxygenation status.

4. Experimental Section

4.1. Chemistry

All anhydrous solvents and reagents used in this study were purchased from Alfa Aesar, TCI, and Sigma-Aldrich. The synthetic reactions involving air- or moisture-sensitive chemicals were carried out under a nitrogen atmosphere using dried glassware and syringe techniques in order to transfer the solutions. Nuclear magnetic resonance (^{1} H-, ^{13}C-, and ^{19}F-NMR) spectra were recorded using a Bruker Avance III 400 MHz spectrometer using DMSO-d_6 as solvent. The chemical shifts are reported in parts per million (ppm), and the coupling constants (*J*) are expressed in Hertz (Hz). The splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; dd, doublet of doublets. The correct assignment of exchangeable protons (i.e., O*H* and N*H*) was carried out by means of the addition of D_2O. Analytical thin-layer chromatography (TLC) was done on Merck silica gel F-254 plates. The HPLC was performed by using a Waters 2690 separation module coupled with a photodiode array detector (PDA Waters 996) using a Nova-Pak C18 4 μm 3.9 mm × 150 mm (Waters) silica-based reverse phase column. The sample was dissolved in 10% acetonitrile/H_2O and an injection volume of 45 μL. The mobile phase (flow rate 1.0 mL/min) was a gradient of H_2O + trifluoroacetic acid (TFA) 0.1% (A) and acetonitrile + TFA 0.1% (B), with steps as follows: (A%/B%), 0−10 min 90:10, 10−25 min gradient to 60:40, 26:28 min isocratic 20:80, 29−35 min isocratic 90:10. TFA 0.1% in water as well in acetonitrile was used as counterion. All compounds reported here were ≥95% HPLC pure. The solvents used in MS measures were acetone, acetonitrile (Chromasolv grade), and mQ water 18 MU. The high resolution mass spectrometry (HRMS) analysis was performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer coupled with an electrospray ionization source (ESI). Analysis was carried out in positive ion mode $[M + H]^+$, and it was used a proper dwell time acquisition to achieve 60,000 units of resolution at full width at half maximum (fwhm). Elemental composition of compounds was calculated on the basis of their measured accurate masses, accepting only results with an attribution error less than 5 ppm and a not integer RDB (double bond/ring equivalents) value. tock solutions of analytes were prepared using acetone (1.0 mg mL^{-1}) and stored at 4 °C. Then working solutions of each analyte were prepared by dilution of the stock solutions using mQ H_2O/ACN 1/1 (*v/v*) up to a concentration of 1.0 μg mL^{-1}. The HRMS analysis was performed by introducing the analyte working solution via syringe pump at 10 μL min^{-1}.

4.2. Synthesis of reported compounds

4.2.1. General synthetic procedure **a** to synthesis of urea derivatives **12-21**

A solution of amine **1-10** (0.2g, 1 equiv) was treated with 4-chloro-3-(trifluoromethyl)phenyl isocyanate **11** (1.0 equv) in dry acetonitrile (3-4 mL). The reaction mixture was stirred at rt until the consumption of starting materials (TLC monitoring). Reaction was quenched with H_2O to give a precipitate that was filtered-off and washed with diethyl ether (3 x 5 mL) and dried under vacuum to afford the products **12-21**.

4.2.2. General synthetic procedure **b** to synthesis of sulfamates **23-24**

A solution of phenols **18-19** (0.2 g, 1.0 equiv) was treated with freshly prepared sulfamoylchloride **22** (6.0 equiv, portion-wise) in dry DMA at 0 °C. The reaction mixture was warmed to rt and stirred for 15 min then quenched with slush. The obtained precipitate was filtered-off, washed with DCM (3 x 5 mL) and dried under vacuum to afford desired products **23-24**.

4.2.3 Synthesis of 2,5-dioxopyrrolidin-1-yl4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido) benzoate *(**25**):* A solution of **20** (1.0 g, 1.0 equiv) was treated with NHS (1.5 equiv) at 0 ˚C in dry DMF (5.0 ml), followed by addition of EDCI (1.5 equiv). The reaction mixture was stirred until the consumption of starting materials (TLC monitoring). The reaction was quenched with H_2O to obtain a precipitate which was filtered-off, washed with H_2O (3 x 5 mL) and dried under vacuum to obtain compound **25**.

4.2.4. General synthetic method **c** for the synthesis of compounds **26-27**, **29**, **31**, **33**, **35**: A solution of amine **5-6**, **28**, **30**, **32** and **34** (50-150 mg, 1.0 equiv) in dry DMF (3-4 mL) was treated with activated ester **25** (1.0 equiv), followed by addition of Et_3N (2.5 equiv). The reaction mixture was stirred until the consumption of the starting materials, then quenched with H_2O (2-3 mL) and acidified by 1M aqueous solution of HCl. The obtained precipitate was filtered-off, washed with diethyl ether (3 x 5 mL) and dried under vacuum to obtain desired products **26-27**, **29, 31**, **33** and **35**.

4.2.5. *5-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-1,3,4-thiadiazole-2-sulfonamide (**12**):* white solid; yield 65%; silica gel TLC R_f 0.35 (MeOH/DCM 10% *v/v*); mp 242-243 °C; δ_H (400 MHz, DMSO-d_6) 7.72 (1H, d, *J* 8.8), 7.83 (1H, m), 8.13 (1H, s), 8.35 (2H, exchange with D_2O, SO_2NH_2), 9.72 (1H, s, exchange with D_2O, N*H*), 11.88 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 118.9 (q, $^3J_{C\text{-}F}$ 6), 123.6 (1, $^1J_{C\text{-}F}$ 271), 125.0, 125.1, 127.7 (q, $^2J_{C\text{-}F}$ 30), 133.1, 138.8, 153.3, 164.1, 164.5; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 399.9 $[M\text{-}H]^-$.

4.2.6. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)benzenesulfonamide* (***13***): White solid, yield 60%; silica gel TLC R_f 0.55 (MeOH/DCM 20% *v/v*); mp 260-261 °C; δ_H (400 MHz, DMSO-d_6) 7.26 (2H, s, exchange with D_2O, SO_2NH_2), 7.66 (4H, m), 7.77 (2H, d, *J* 8.0), 8.16 (1H, m), 9.28 (1H, s,

exchange with D_2O, N*H*), 9.31 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 117.9 (q, $^3J_{C\text{-}F}$6), 118.9, 123.7 (q, $^4J_{C\text{-}F}$ 2), 123.8 (q, $^1J_{C\text{-}F}$ 271.3), 124.2, 127.7 (q, $^2J_{C\text{-}F}$ 30.4), 127.8, 133.0, 138.3, 139.9, 143.3, 153.2; δ_F (376 MHz, DMSO-d_6) -61.4; *m/z* (ESI negative) 392.0 $[M\text{-}H]^-$.

4.2.7. *3-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)benzenesulfonamide* ***(14)***: White solid, yield 76%; silica gel TLC R_f 0.45 (MeOH/DCM 10% *v/v*); mp 245-246 ºC; δ_H (400 MHz, DMSO-d_6) 7.40 (2H, s, exchange with D_2O, SO_2NH_2), 7.51 (2H, m), 7.60 (1H, dt, *J* 2.0, 7.6), 7.68 (2H, m), 8.12 (1H, m), 8.17 (1H, d, *J* 2.0), 9.23 (1H, s, exchange with D_2O, N*H*), 9.25 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 116.5, 117.9 (q, $^3J_{C\text{-}F}$ 22), 120.3, 122.5, 123.6 (q, $^4J_{C\text{-}F}$ 2), 123.8 (q, $^1J_{C\text{-}F}$ 271), 124.2, 127.7 (q, $^3J_{C\text{-}F}$ 30), 130.4, 132.9, 140.1, 140.7, 145.7, 153.3; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI positive) 392.0 $[M\text{-}H]^-$.

4.2.8. *3-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-4-hydroxybenzenesulfonamide* ***(15)***: White solid, yield 62%; silica gel TLC R_f 0.12 (MeOH/DCM 10% *v/v*); mp 254-255 ºC; δ_H (400 MHz, DMSO-d_6) 6.99 (1H, d, *J* 8.4), 7.16 (2H, s, exchange with D_2O, SO_2NH_2), 7.36 (1H, dd, *J* 2.4, 8.4), 7.60 (1H, dd, *J* 2.0, 8.8), 7.66 (1H, d, *J* 8.8), 8.20 (1H, d, *J* 2.0), 8.48 (1H, s, exchange with D_2O, N*H*), 8.66 (1H, d, *J* 2.4), 9.89 (1H, s, exchange with D_2O, N*H*), 10.96 (1H, s, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 114.7, 117.1, 117.3 (q, $^3J_{C\text{-}F}$ 6), 121.4, 123.2 (q, $^1J_{C\text{-}F}$ 273), 123.4 (q, $^4J_{C\text{-}F}$ 2), 123.6, 127.8 (q, $^2J_{C\text{-}F}$ 30), 128.3, 133.1, 135.9, 140.2, 149.5, 153.1; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 408.0 $[M\text{-}H]^-$.

4.2.9. *4-((3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)methyl)benzenesulfonamide* ***(16)***: White solid, yield 20%; mp 158-159 °C; δ_H (400 MHz, DMSO-d_6) 4.40 (2H, d, *J* 5.6), 7.00 (1H, t, *J* 5.6, exchange with D_2O, N*H*), 7.34 (2H, s, exchange with D_2O, SO_2NH_2), 7.50 (2H, d, *J* 8.4), 7.58 (1H, d, *J* 8.8), 7.64 (1H, dd, *J* 2.4, 8.8), 7.81 (2H, d, *J* 8.4), 8.11 (1H, d, *J* 2.4), 9.25 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 43.4, 117.1 (q, $^3J_{C\text{-}F}$ 6), 122.4 (q, $^4J_{C\text{-}F}$ 2), 123.2, 123.8 (q, $^1J_{C\text{-}F}$ 271), 126.7, 127.6 (q, $^2J_{C\text{-}F}$ 30), 128.2, 132.7, 141.0, 143.5, 145.2, 155.9; δ_F (376 MHz, DMSO-d_6) -61.4; *m/z* (ESI positive) 452.0 $[M+HCOO]^-$.

4.2.10. *4-(2-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)ethyl)benzenesulfonamide* ***(17)***: White solid, yield 78%; silica gel TLC R_f 0.67 (MeOH/DCM 20% *v/v*); mp 229-230 °C; δ_H (400 MHz, DMSO-d_6) 2.88 (2H, t, *J* 6.8), 3.41 (2H, q, *J* 6.8), 6.39 (1H, t, *J* 6.8, exchange with D_2O, N*H*), 7.33 (2H, s, exchange with D_2O, SO_2NH_2), 7.46 (2H, d, *J* 8.2), 7.58 (2H, m), 7.79 (2H, d, *J* 8.2), 8.10 (1H, m), 9.03 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 36,3, 41.3, 117.1 (q, $^3J_{C\text{-}F}$ 6), 122.4 (q, $^4J_{C\text{-}F}$ 2), 123.2, 123.8 (q, $^1J_{C\text{-}F}$ 271), 126.7, 127.6 (q, $^2J_{C\text{-}F}$ 30), 130.1, 132.7, 141.0, 143.1, 144.7, 155.8; δ_F (376 MHz, DMSO-d_6) -61.4; *m/z* (ESI positive) 422.0 $[M+H]^+$.

4.2.11. *1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-hydroxyphenyl)urea (**18**)*: White solid, yield 55%; silica gel TLC R_f 0.33 (MeOH/DCM 10% *v/v*); δ_H (400 MHz, DMSO-d_6) 6.73 (2H, d, *J* 8.8), 7.26 (2H, d, *J* 8.8), 7.64 (2H, m), 8.13 (1H, d, *J* 2.4), 8.51 (1H, exchange with D_2O, N*H*), 9.05 (1H, exchange with D_2O, N*H*), 9.15 (1H, s, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 116.2, 117.5 (q, $^3J_{C\text{-}F}$ 6), 122.0, 122.9 (q, $^4J_{C\text{-}F}$ 2), 123.7, 123.8 (q, $^1J_{C\text{-}F}$ 271), 127.8 (q, $^2J_{C\text{-}F}$ 30), 131.5, 132.8, 140.6, 153.6, 154.0; δ_F (376 MHz, DMSO-d_6) -61.5.

4.2.12. *1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(3-hydroxyphenyl)urea (**19**)*: White solid, yield 60%; silica gel TLC R_f 0.47 (MeOH/DCM 10% *v/v*); δ_H (400 MHz, DMSO-d_6) 6.44 (1H, m), 6.84 (1H, m), 7.08 (2H, m), 7.64 (1H, d, *J* 1.2), 8.15 (1H, t, *J* 1.2), 8.75 (1H, exchange with D_2O, N*H*), 9.10 (1H, exchange with D_2O, N*H*), 9.37 (1H, s, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 106.6, 110.2, 110.4, 117.6 (q, $^3J_{C\text{-}F}$ 6), 123.1 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 271), 123.9, 127.5 (q, $^2J_{C\text{-}F}$ 30), 130.4, 132.9, 140.3, 141.1, 153.2, 158.7; δ_F (376 MHz, DMSO-d_6) -61.5.

4.2.13. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)benzoic acid (**20**)*: White solid, yield 77%; silica gel TLC R_f 0.45 (EtOAc/*n*-hexane 80% *v/v*); δ_H (400 MHz, DMSO-d_6) 7.62 (2H, d, *J* 8.8), 7.68 (2H, m), 7.91 (2H, d, *J* 8.8), 8.15 (1H, m), 9.26 (1H, exchange with D_2O, N*H*), 9.30 (1H, s, exchange with D_2O, N*H*), 12.68 (1H, s, exchange with D_2O, COO*H*); δ_C (100 MHz, DMSO-d_6) 117.9 (q, $^3J_{C\text{-}F}$ 6), 118.6, 123.6 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 271), 124.2, 125.1, 127.7 (q, $^2J_{C\text{-}F}$ 30), 131.5, 133.0, 140.0, 144.5, 153.1, 167.9; δ_F (376 MHz, DMSO-d_6) -61.5.

4.2.14. *1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-methyl-2-oxo-2H-chromen-7-yl)urea (**21**)*: White solid; yield 70%; mp>300°C; δ_H (400 MHz, DMSO-d_6) 2.44 (3H, s), 6.27 (1H, s), 7.40 (1H, d, *J* 8.6), 7.64-7.74 (4H, m), 8.13 (1H, m), 9.39 (1H, s, exchange with D_2O, N*H*), 9.42 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 18.8, 105.6, 112.6, 115.0, 115.4, 117.8 (q, $^3J_{C\text{-}F}$ 6), 123.5 (q, $^4J_{C\text{-}F}$ 2), 123.6 (q, $^1J_{C\text{-}F}$ 270), 124.2, 126.7, 127.7 (q, $^2J_{C\text{-}F}$ 30), 132.9, 139.8, 143.8, 153.0, 154.0, 154.8, 160.9; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 395.0 $[M\text{-}H]^-$.

4.2.15. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl sulfamate (**23**)*: White solid, yield 77%; silica gel TLC R_f 0.70 (MeOH/DCM 10% *v/v*); mp 175-176°C (dec); δ_H (400 MHz, DMSO-d_6) 7.24 (2H, d, *J* 9.0), 7.55 (2H, d, *J* 9.0), 7.66 (2H, m), 7.95 (2H, s, exchange with D_2O, SO_2NH_2), 8.14 (1H, d, *J* 2.2), 9.00 (1H, exchange with D_2O, N*H*), 9.21 (1H, exchange with D_2O, N*H*);δ_C (100 MHz, DMSO-d_6) 117.7 (q, $^3J_{C\text{-}F}$ 6), 120.6, 123.3 (q, $^4J_{C\text{-}F}$ 3), 123.6, 123.8 (q, $^1J_{C\text{-}F}$ 271), 124.0, 127.7 (q, $^2J_{C\text{-}F}$ 30), 132.9, 138.6, 140.2, 145.9, 153.4; *m/z* (ESI negative) 408.0 $[M\text{-}H]^-$.

4.2.16. *3-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl sulfamate (**24**)*: White solid, yield 65%; silica gel TLC R_f 0.55 (MeOH/DCM 10% *v/v*); mp 190-191°C (dec); δ_H (400 MHz, DMSO-d_6) 6.95 (1H, m), 7.39 (2H, m), 7.57 (1H, m), 7.67 (2H, m), 8.03 (2H, exchange with D_2O, SO_2NH_2),

8.15 (1H, d, *J* 2.0), 9.11 (1H, exchange with D_2O, N*H*), 9.21 (1H, s, exchange with D_2O, N*H*);δ_C (100 MHz, DMSO-d_6) 113.2, 116.6, 117.4, 117.8 (q, $^3J_{C\text{-}F}$ 6), 123.4 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 271), 124.1, 127.7 (q, $^2J_{C\text{-}F}$ 30), 130.7, 132.9, 140.1, 141.4, 151.4, 153.2; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 408.0 $[M\text{-}H]^-$.

4.2.17. *2,5-dioxopyrrolidin-1-yl 4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)benzoate (**25**):* White solid; yield 92%; δ_H (400 MHz, DMSO-d_6) 2.92 (4H, s), 7.70 (2H, m), 7.77 (2H, d, *J* 9.0), 7.99 (1H, s), 8.07 (2H, d, *J* 9.0), 8.17 (1H, d, *J* 2.2), 9.45 (1H, exchange with D_2O, N*H*), 9.57 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 26.4, 118.1, 118.1 (q, $^3J_{C\text{-}F}$ 6), 119.0, 123.8 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 271), 124.4, 127.7 (q, $^2J_{C\text{-}F}$ 30), 132.4, 132.9, 139.7, 146.8, 152.9, 162.2, 171.3; δ_F (376 MHz, DMSO-d_6) -61.5.

4.2.18. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(4-sulfamoylbenzyl)benzamide (**26**)*: White solid; yield 60%; silica gel TLC R_f0.26 (MeOH/DCM 10% *v/v*); mp195-196 °C; δ_H (400 MHz, DMSO-d_6) 4.56 (2H, d, *J* 6.0), 7.35 (2H, s, exchange with D_2O, SO_2NH_2), 7.52 (2H, d, *J* 8.3), 7.59 (2H, d, *J* 8.8), 7.68 (2H, m), 7.81 (2H, d, *J* 8.3), 7.90 (2H, d, *J* 8.8), 8.16 (1H, m), 9.04 (1H, t, *J* 6.0, exchange with D_2O, N*H*), 9.18 (1H, s, exchange with D_2O, N*H*), 9.30 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 43.2, 117.8 (q, $^3J_{C\text{-}F}$ 6), 118.5, 123.5 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 270), 124.2, 126.6, 127.7 (q, $^2J_{C\text{-}F}$ 30), 128.4, 128.6, 129.2, 133.0, 140.1, 143.1, 143.5, 144.9, 153.2, 166.8; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 525.1 $[M\text{-}H]^-$.

4.2.19. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(4-sulfamoylphenethyl)benzamide (**27**)*: White solid; yield 62%; silica gel TLC R_f0.22 (EtOAc/*n*-hexane 80% *v/v*); mp 269-270 °C; δ_H (400 MHz, DMSO-d_6) 2.96 (2H, t, *J* 7.2), 3.54 (2H, q, *J* 7.2), 7.32 (2H, s, exchange with D_2O, SO_2NH_2), 7.47 (2H, d, *J* 8.4), 7.57 (2H, d, *J* 8.8), 7.67 (2H, m), 7.80 (4H, m), 8.15 (1H, m), 8.49 (1H, t, *J* 7.2, exchange with D_2O, N*H*), 9.14 (1H, s, exchange with D_2O, N*H*), 9.29 (1H, s, exchange with D_2O, N*H*);δ_C (100 MHz, DMSO-d_6) 35.8, 41.3, 117.8 (q, $^3J_{C\text{-}F}$ 6), 118.5, 123.5 (q, $^4J_{C\text{-}F}$ 2), 123.7 (q, $^1J_{C\text{-}F}$ 270), 124.1, 126.6, 127.7 (q, $^2J_{C\text{-}F}$ 30), 129.0, 129.0, 130.1, 133.0, 140.1, 142.8, 143.0, 144.8, 153.2, 166.7; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 539.1 $[M\text{-}H]^-$.

4.2.20. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(6-(4-sulfamoylphenoxy)hexyl)benzamide (**29**):* White solid, yield 64%; silica gel TLC R_f 0.25 (MeOH/DCM 10% *v/v*); mp 231-232 °C; δ_H (400 MHz, DMSO-d_6) 2.03 (2H, pent, *J* 6.5), 3.45 (2H, q, *J* 6.5), 4.15 (2H, t, *J* 6.5), 7.12 (2H, d, *J* 8.9), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.57 (2H, d, *J* 8.8), 7.67 (2H, m), 7.78 (2H, d, *J* 8.9), 7.84 (2H, d, *J* 8.8), 8.15 (1H, m), 8.46 (1H, t, *J* 6.5, exchange with D_2O, N*H*), 9.14 (1H, s, exchange with D_2O, N*H*), 9.27 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 29.8, 37.1, 66.8, 115.4, 117.8 (q, $^3J_{C\text{-}F}$ 6), 118.4, 123.5 (q, $^4J_{C\text{-}F}$ 2),

123.7 (q, $^{1}J_{C\text{-}F}$ 270), 124.1, 127.7 (q, $^{2}J_{C\text{-}F}$ 30), 127.8, 128.6, 129.0, 129.1, 132.9, 137.0, 140.1, 142.8, 153.2, 162.0, 166.8; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 569.1 $[M\text{-}H]^-$.

4.2.21. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(6-(4-sulfamoylphenoxy)hexyl)benzamide **(31)**:* White solid; yield 55%; mp 237-238°C; δ_H (400 MHz, DMSO-d_6) 1.44 (4H, m), 1.58 (2H, pent, *J* 7.0), 1.77 (2H, pent, *J* 7),3.28 (2H, q, *J* 7.0), 4.08 (2H, t, *J* 7.0), 7.10 (2H, d, *J* 8.8), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.57 (2H, d, *J* 8.6), 7.66 (2H, m), 7.78 (2H, d, *J* 8.8), 7.83 (2H, d, *J* 8.6), 8.16 (1H, s), 8.36 (1H, t, *J* 7.0, exchange with D_2O, N*H*), 9.15 (1H, s, exchange with D_2O, N*H*), 9.30 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 26.2, 27.2, 29.4, 30.1, 68.8, 115.3,117.8 (q, $^{3}J_{C\text{-}F}$ 6), 118.4, 123.5 (q, $^{4}J_{C\text{-}F}$ 2), 123.7 (q, $^{1}J_{C\text{-}F}$ 270), 124.1, 127.7 (q, $^{2}J_{C\text{-}F}$ 30), 127.8, 128.6, 129.0, 129.2, 133.0, 136.9, 140.1, 142.7, 153.2, 162.0, 166.5; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 611.2 $[M\text{-}H]^-$.

4.2.22. 4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(2-((2-oxo-2H-chromen-7-yl)oxy)ethyl) benzamide *(**33**)*: White solid; yield 35%; mp 217-218 °C; δ_H (400 MHz, DMSO-d_6) 3.67 (2H, q, *J* 5.6), 4.26 (2H, t, *J* 5.6), 6.32 (1H, d, *J* 9.5), 7.00 (1H, dd, *J* 2.4, 8.6), 7.08 (1H, d, *J* 2.4), 7.57 (2H, d, *J* 8.8), 7.66 (3H, m), 7.86 (2H, d, *J* 8.8), 8.02 (1H, d, *J* 9.5), 8.15 (1H, m), 8.65 (1H, t, *J* 5.6, exchange with D_2O, N*H*), 9.21 (1H, s, exchange with D_2O, N*H*), 9.36 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 39.5, 67.8, 102.2, 113.4, 113.5, 113.7, 117.7 (q, $^{3}J_{C\text{-}F}$ 6), 118.4, 123.5 (q, $^{4}J_{C\text{-}F}$ 2), 123.8 (q, $^{1}J_{C\text{-}F}$ 270), 124.1, 127.6 (q, $^{2}J_{C\text{-}F}$ 30), 127.8, 128.6, 128.6, 129.2, 130.5, 133.0, 140.1, 143.1, 145.3, 153.2, 156.4, 161.3, 162.6, 167.0; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 544.1 $[M\text{-}H]^-$.

4.2.23. *4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-N-(2-((2-oxo-2H-chromen-6-yl)oxy)ethyl) benzamide (**35**)*: White solid, yield 42%; mp 219-220 °C; δ_H (400 MHz, DMSO-d_6) 3.68 (2H, q, *J* 5.6), 4.20 (2H, t, *J* 5.6), 6.53 (1H, d, *J* 9.6), 7.27 (1H, dd, *J* 2.4, 8.8), 7.38 (2H, m), 7.58 (2H, d, *J* 8.8), 7.67 (2H, m), 7.86 (2H, d, *J* 8.8), 8.04 (1H, d, *J* 9.6), 8.15 (1H, m), 8.66 (1H, t, *J* 5.6,exchange with D_2O, N*H*), 9.29 (1H, s, exchange with D_2O, N*H*), 9.45 (1H, s, exchange with D_2O, N*H*); δ_C (100 MHz, DMSO-d_6) 39.7, 67.7, 112.5, 117.5, 117.7 (q, $^{3}J_{C\text{-}F}$ 6), 118.3, 118.4, 120.2, 120.8, 123.4 (q, $^{4}J_{C\text{-}F}$ 2), 124.0, 124.2 (q, $^{1}J_{C\text{-}F}$ 270), 127.6 (q, $^{2}J_{C\text{-}F}$ 30), 128.6, 129.2, 133.0, 140.1, 143.0, 145.0, 148.8,153.2, 155.7, 161.1, 167.0 ; δ_F (376 MHz, DMSO-d_6) -61.5; *m/z* (ESI negative) 544.1 $[M\text{-}H]^-$.

4.3. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [52]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2

hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together at room temperature (15 min) prior to assay, in order to allow for the formation of the E-I complex. Data from Table 1 were obtained after 15 minutes incubation of enzyme and inhibitor, as for the sulfonamides reported earlier [5,42,55,56]. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier [5,42,55,56] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier [5,42,55,56].

4.4. Co-crystallization and X-ray data collection

Crystals of hCA I complexed with compounds **17** and **23** were obtained using the sitting drop vapor diffusion method. 2 µl of 10 mg/ml solution of hCA I in Tris-HCl 20 mM pH 9.0 were mixed with 2 µl of a solution of 28-31% PEG4000, 0.2 M Sodium acetate, 0.1 M Tris pH 8.5-9.0 and were equilibrated against the same solution at 296 K. Crystals of the protein grew in fifteen days. Afterwards hCAI crystals were soaked in 5mM inhibitor solutions for 3 days.

The crystals were flash-frozen at 100 K using a solution obtained by adding 15% (v/v) glycerol to the mother liquor solution as cryoprotectant. Data on crystals of the complexes were collected using synchrotron radiation at the ID30A-1 beamline at ESRF (Grenoble, France) with a wavelength of 0.966 Å and a PILATUS3 2M Dectris CCD detector. Data were integrated and scaled using the program XDS [57].Data processing statistics are shown in Supporting Information Table S1.

4.4.1. Structure determination

The crystal structure of hCA I (PDB accession code 1JV0) without solvent molecules and other heteroatoms was used to obtain initial phases of the structures using Refmac5 [58]. 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of R_{free} calculations. The initial |Fo - Fc| difference electron density map unambiguously showed the inhibitor molecules. Atomic models for inhibitors were calculated and energy minimized using the program JLigand 1.0.40 [59]. Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT [60]. Solvent molecules were introduced automatically using the program ARP [61]. The quality of the final models

were assessed with COOT and RAMPAGE [62]. Crystal parameters and refinement data are summarized in Table S1 in the Supporting information. Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 6I0L and 6I0J). Graphical representations were generated with Chimera [63].

4.5. Cell Lines and Culture

The breast cancer cell lines, HBL100, MCF7 and MDA.MB.231 were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin in 21% O_2. Hypoxic cells were grown at 0.5% O_2 in a Don Whitley H35 Hypoxystation. All cell lines used were tested and authenticated using STR profiling by Public Health England, Porton Down, Salisbury, UK.

4.5.1. Sulforhodamine B assay

Cells (1 x 10^3/well) were seeded into 96-well flat-bottomed plates and incubated for 48 h before the sulfamates were added using concentrations between 0 – 300 µM. After 5 days treatment, plates were fixed by the addition of 50 µl 25% trichloroacetic acid solution per well for 1 h at 4 °C. Plates were washed with H_2O and air dried before the addition of 50 µl 0.4% sulforhodamine B solution in 1% acetic acid for 30 min. Plates were washed in 1% acetic acid and air dried before the addition of 150 µl 10 mM Tris solution (pH 10.5). After 30 min, the absorbance of each well was measured at 540 nm on a BP900 biohit plate reader.

References

[1] C.T. Supuran, Carbonic anhydrases, Bioorg. Med. Chem. 21 (2013) 1377–1378. doi:10.1016/j.bmc.2013.02.026.

[2] Structure and function of carbonic anhydrases, Biochem. J. 473 (2016) 2023–2032. doi:10.1042/BCJ20160115.

[3] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat Rev Drug Discov. 7 (2008) 168–181. doi:10.1038/nrd2467.

[4] D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat Rev Drug Discov. 10 (2011) 767–777. doi:10.1038/nrd3554.

[5] M. Abdoli, A. Angeli, M. Bozdag, F. Carta, A. Kakanejadifard, H. Saeidian, C.T. Supuran, Synthesis and carbonic anhydrase I, II, VII, and IX inhibition studies with a series of benzo[d]thiazole-5- and 6-sulfonamides, J Enzyme Inhib Med Chem. 32 (2017) 1071–1078. doi:10.1080/14756366.2017.1356295.

[6] M. Bozdag, F. Carta, D. Vullo, A. Akdemir, S. Isik, C. Lanzi, A. Scozzafava, E. Masini, C.T. Supuran, Synthesis of a new series of dithiocarbamates with effective human carbonic anhydrase inhibitory activity and antiglaucoma action, Bioorg. Med. Chem. 23 (2015) 2368–2376. doi:10.1016/j.bmc.2015.03.068.

[7] F. Carta, M. Aggarwal, A. Maresca, A. Scozzafava, R. McKenna, C.T. Supuran, Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations, Chem. Commun. (Camb.). 48 (2012) 1868–1870. doi:10.1039/c2cc16395k.

[8] F. Carta, M. Aggarwal, A. Maresca, A. Scozzafava, R. McKenna, E. Masini, C.T. Supuran, Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action in vivo, J. Med. Chem. 55 (2012) 1721–1730. doi:10.1021/jm300031j.
[9] S. Avram, A.L. Milac, F. Carta, C.T. Supuran, More effective dithiocarbamate derivatives inhibiting carbonic anhydrases, generated by QSAR and computational design, J Enzyme Inhib Med Chem. 28 (2013) 350–359. doi:10.3109/14756366.2012.727410.
[10] F. Carta, A. Akdemir, A. Scozzafava, E. Masini, C.T. Supuran, Xanthates and trithiocarbonates strongly inhibit carbonic anhydrases and show antiglaucoma effects in vivo, J. Med. Chem. 56 (2013) 4691–4700. doi:10.1021/jm400414j.
[11] D. Vullo, M. Durante, F.S. Di Leva, S. Cosconati, E. Masini, A. Scozzafava, E. Novellino, C.T. Supuran, F. Carta, Monothiocarbamates Strongly Inhibit Carbonic Anhydrases in Vitro and Possess Intraocular Pressure Lowering Activity in an Animal Model of Glaucoma, J. Med. Chem. 59 (2016) 5857–5867. doi:10.1021/acs.jmedchem.6b00462.
[12] A. Innocenti, D. Vullo, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: interactions of phenols with the 12 catalytically active mammalian isoforms (CA I-XIV), Bioorg. Med. Chem. Lett. 18 (2008) 1583–1587. doi:10.1016/j.bmcl.2008.01.077.
[13] R.A. Davis, A. Hofmann, A. Osman, R.A. Hall, F.A. Mühlschlegel, D. Vullo, A. Innocenti, C.T. Supuran, S.-A. Poulsen, Natural product-based phenols as novel probes for mycobacterial and fungal carbonic anhydrases, J. Med. Chem. 54 (2011) 1682–1692. doi:10.1021/jm1013242.
[14] A. Innocenti, I. Gülçin, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I-XV, Bioorg. Med. Chem. Lett. 20 (2010) 5050–5053. doi:10.1016/j.bmcl.2010.07.038.
[15] A. Innocenti, D. Vullo, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: inhibition of mammalian isoforms I-XIV with a series of substituted phenols including paracetamol and salicylic acid, Bioorg. Med. Chem. 16 (2008) 7424–7428. doi:10.1016/j.bmc.2008.06.013.
[16] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms?, Chem. Rev. 112 (2012) 4421–4468. doi:10.1021/cr200176r.
[17] E. Langella, K. D'Ambrosio, M. D'Ascenzio, S. Carradori, S.M. Monti, C.T. Supuran, G. De Simone, A Combined Crystallographic and Theoretical Study Explains the Capability of Carboxylic Acids to Adopt Multiple Binding Modes in the Active Site of Carbonic Anhydrases, Chemistry. 22 (2016) 97–100. doi:10.1002/chem.201503748.
[18] A. Scozzafava, C.T. Supuran, F. Carta, Polyamines and α-Carbonic Anhydrases, Molecules. 21 (2016). doi:10.3390/molecules21121726.
[19] F. Carta, C. Temperini, A. Innocenti, A. Scozzafava, K. Kaila, C.T. Supuran, Polyamines inhibit carbonic anhydrases by anchoring to the zinc-coordinated water molecule, J. Med. Chem. 53 (2010) 5511–5522. doi:10.1021/jm1003667.
[20] F. Carta, A. Maresca, A. Scozzafava, C.T. Supuran, Novel coumarins and 2-thioxo-coumarins as inhibitors of the tumor-associated carbonic anhydrases IX and XII, Bioorg. Med. Chem. 20 (2012) 2266–2273. doi:10.1016/j.bmc.2012.02.014.
[21] M. Ferraroni, F. Carta, A. Scozzafava, C.T. Supuran, Thioxocoumarins Show an Alternative Carbonic Anhydrase Inhibition Mechanism Compared to Coumarins, J. Med. Chem. 59 (2016) 462–473. doi:10.1021/acs.jmedchem.5b01720.
[22] M. Tanc, F. Carta, M. Bozdag, A. Scozzafava, C.T. Supuran, 7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors, Bioorg. Med. Chem. 21 (2013) 4502–4510. doi:10.1016/j.bmc.2013.05.032.
[23] K. Tars, D. Vullo, A. Kazaks, J. Leitans, A. Lends, A. Grandane, R. Zalubovskis, A. Scozzafava, C.T. Supuran, Sulfocoumarins (1,2-benzoxathiine-2,2-dioxides): a class of potent and isoform-selective inhibitors of tumor-associated carbonic anhydrases, J. Med. Chem. 56 (2013) 293–300. doi:10.1021/jm301625s.

[24] A. Grandane, M. Tanc, L. Di Cesare Mannelli, F. Carta, C. Ghelardini, R. Žalubovskis, C.T. Supuran, 6-Substituted sulfocoumarins are selective carbonic anhdydrase IX and XII inhibitors with significant cytotoxicity against colorectal cancer cells, J. Med. Chem. 58 (2015) 3975–3983. doi:10.1021/acs.jmedchem.5b00523.
[25] A. Grandane, M. Tanc, R. Žalubovskis, C.T. Supuran, Synthesis of 6-aryl-substituted sulfocoumarins and investigation of their carbonic anhydrase inhibitory action, Bioorg. Med. Chem. 23 (2015) 1430–1436. doi:10.1016/j.bmc.2015.02.023.
[26] A. Grandane, M. Tanc, R. Zalubovskis, C.T. Supuran, 6-Triazolyl-substituted sulfocoumarins are potent, selective inhibitors of the tumor-associated carbonic anhydrases IX and XII, Bioorg. Med. Chem. Lett. 24 (2014) 1256–1260. doi:10.1016/j.bmcl.2014.01.076.
[27] A. Grandane, M. Tanc, R. Zalubovskis, C.T. Supuran, Synthesis of 6-tetrazolyl-substituted sulfocoumarins acting as highly potent and selective inhibitors of the tumor-associated carbonic anhydrase isoforms IX and XII, Bioorg. Med. Chem. 22 (2014) 1522–1528. doi:10.1016/j.bmc.2014.01.043.
[28] A. Nocentini, F. Carta, M. Tanc, S. Selleri, C.T. Supuran, C. Bazzicalupi, P. Gratteri, Deciphering the Mechanism of Human Carbonic Anhydrases Inhibition with Sulfocoumarins: Computational and Experimental Studies, Chemistry. 24 (2018) 7840–7844. doi:10.1002/chem.201800941.
[29] A. Maresca, C. Temperini, H. Vu, N.B. Pham, S.-A. Poulsen, A. Scozzafava, R.J. Quinn, C.T. Supuran, Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors, J. Am. Chem. Soc. 131 (2009) 3057–3062. doi:10.1021/ja809683v.
[30] A. Maresca, C. Temperini, L. Pochet, B. Masereel, A. Scozzafava, C.T. Supuran, Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins, J. Med. Chem. 53 (2010) 335–344. doi:10.1021/jm901287j.
[31] M. Bozdag, A.M. Alafeefy, A.M. Altamimi, D. Vullo, F. Carta, C.T. Supuran, Coumarins and other fused bicyclic heterocycles with selective tumor-associated carbonic anhydrase isoforms inhibitory activity, Bioorganic & Medicinal Chemistry. 25 (2017) 677–683. doi:10.1016/j.bmc.2016.11.039.
[32] S. Bua, L. Di Cesare Mannelli, D. Vullo, C. Ghelardini, G. Bartolucci, A. Scozzafava, C.T. Supuran, F. Carta, Design and Synthesis of Novel Nonsteroidal Anti-Inflammatory Drugs and Carbonic Anhydrase Inhibitors Hybrids (NSAIDs-CAIs) for the Treatment of Rheumatoid Arthritis, J. Med. Chem. 60 (2017) 1159–1170. doi:10.1021/acs.jmedchem.6b01607.
[33] K. D'Ambrosio, S. Carradori, S.M. Monti, M. Buonanno, D. Secci, D. Vullo, C.T. Supuran, G. De Simone, Out of the active site binding pocket for carbonic anhydrase inhibitors, Chem. Commun. (Camb.). 51 (2015) 302–305. doi:10.1039/c4cc07320g.
[34] A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: ureido and thioureido derivatives of aromatic sulfonamides possessing increased affinities for isozyme I. A novel route to 2,5-disubstituted-1,3,4-thiadiazoles via thioureas, and their interaction with isozymes I, II and IV, J. Enzym. Inhib. 13 (1998) 103–123.
[35] F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran, Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis, J. Med. Chem. 54 (2011) 1896–1902. doi:10.1021/jm101541x.
[36] Y. Lou, P.C. McDonald, A. Oloumi, S. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U. Auf dem Keller, S. Leung, D. Huntsman, B. Clarke, B.W. Sutherland, D. Waterhouse, M. Bally, C. Roskelley, C.M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.-Y. Winum, C.T. Supuran, S. Dedhar, Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors, Cancer Res. 71 (2011) 3364–3376. doi:10.1158/0008-5472.CAN-10-4261.
[37] F. Pacchiano, M. Aggarwal, B.S. Avvaru, A.H. Robbins, A. Scozzafava, R. McKenna, C.T. Supuran, Selective hydrophobic pocket binding observed within the carbonic anhydrase II

active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency, Chem. Commun. (Camb.). 46 (2010) 8371–8373. doi:10.1039/c0cc02707c.
[38] A Study of SLC-0111 and Gemcitabine for Metastatic Pancreatic Ductal Cancer in Subjects Positive for CAIX - Full Text View - ClinicalTrials.gov, (n.d.). https://clinicaltrials.gov/ct2/show/NCT03450018 (accessed January 24, 2019).
[39] P.C. McDonald, J.-Y. Winum, C.T. Supuran, S. Dedhar, Recent Developments in Targeting Carbonic Anhydrase IX for Cancer Therapeutics, Oncotarget. 3 (2012) 84–97. doi:10.18632/oncotarget.422.
[40] M. Hilvo, Expression of Carbonic Anhydrase IX in Mouse Tissues, Journal of Histochemistry and Cytochemistry. 52 (2004) 1313–1322. doi:10.1369/jhc.3A6225.2004.
[41] C.T. Supuran, Carbonic Anhydrase Inhibition and the Management of Hypoxic Tumors, Metabolites. 7 (2017). doi:10.3390/metabo7030048.
[42] J.-Y. Winum, F. Carta, C. Ward, P. Mullen, D. Harrison, S.P. Langdon, A. Cecchi, A. Scozzafava, I. Kunkler, C.T. Supuran, Ureido-substituted sulfamates show potent carbonic anhydrase IX inhibitory and antiproliferative activities against breast cancer cell lines, Bioorg. Med. Chem. Lett. 22 (2012) 4681–4685. doi:10.1016/j.bmcl.2012.05.083.
[43] C. Ward, J. Meehan, P. Mullen, C. Supuran, J.M. Dixon, J.S. Thomas, J.-Y. Winum, P. Lambin, L. Dubois, N.-K. Pavathaneni, E.J. Jarman, L. Renshaw, I.H. Um, C. Kay, D.J. Harrison, I.H. Kunkler, S.P. Langdon, Evaluation of carbonic anhydrase IX as a therapeutic target for inhibition of breast cancer invasion and metastasis using a series of in vitro breast cancer models, Oncotarget. 6 (2015) 24856–24870. doi:10.18632/oncotarget.4498.
[44] M. Bozdag, F. Carta, M. Ceruso, M. Ferraroni, P.C. McDonald, S. Dedhar, C.T. Supuran, Discovery of 4-Hydroxy-3-(3-(phenylureido)benzenesulfonamides as SLC-0111 Analogues for the Treatment of Hypoxic Tumors Overexpressing Carbonic Anhydrase IX, J. Med. Chem. 61 (2018) 6328–6338. doi:10.1021/acs.jmedchem.8b00770.
[45] M. Bozdag, F. Carta, A. Angeli, S.M. Osman, F.A.S. Alasmary, Z. AlOthman, C.T. Supuran, Synthesis of N'-phenyl-N-hydroxyureas and investigation of their inhibitory activities on human carbonic anhydrases, Bioorg. Chem. 78 (2018) 1–6. doi:10.1016/j.bioorg.2018.02.029.
[46] M. Bozdag, G. Poli, A. Angeli, E. Lucarini, T. Tuccinardi, L. Di Cesare Mannelli, S. Selleri, C. Ghelardini, J.-Y. Winum, F. Carta, C.T. Supuran, N-aryl-N'-ureido-O-sulfamates: Potent and selective inhibitors of the human Carbonic Anhydrase VII isoform with neuropathic pain relieving properties, Bioorganic Chemistry. 89 (2019) 103033. doi:10.1016/j.bioorg.2019.103033.
[47] C. Méndez-Blanco, F. Fondevila, A. García-Palomo, J. González-Gallego, J.L. Mauriz, Sorafenib resistance in hepatocarcinoma: role of hypoxia-inducible factors, Experimental & Molecular Medicine. 50 (2018) 134. doi:10.1038/s12276-018-0159-1.
[48] G.M. Keating, A. Santoro, Sorafenib: a review of its use in advanced hepatocellular carcinoma, Drugs. 69 (2009) 223–240. doi:10.2165/00003495-200969020-00006.
[49] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: A Cancer Journal for Clinicians. 68 (2018) 394–424. doi:10.3322/caac.21492.
[50] R. Dutta, R.I. Mahato, Recent advances in hepatocellular carcinoma therapy, Pharmacol. Ther. 173 (2017) 106–117. doi:10.1016/j.pharmthera.2017.02.010.
[51] M. Bozdag, M. Pinard, F. Carta, E. Masini, A. Scozzafava, R. McKenna, C.T. Supuran, A Class of 4-Sulfamoylphenyl-ω-aminoalkyl Ethers with Effective Carbonic Anhydrase Inhibitory Action and Antiglaucoma Effects, J. Med. Chem. 57 (2014) 9673–9686. doi:10.1021/jm501497m.
[52] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem. 246 (1971) 2561–2573.

[53] A. Angeli, F. Carta, G. Bartolucci, C.T. Supuran, Synthesis of novel acyl selenoureido benzensulfonamides as carbonic anhydrase I, II, VII and IX inhibitors, Bioorg. Med. Chem. 25 (2017) 3567–3573. doi:10.1016/j.bmc.2017.05.014.
[54] R.G. Gieling, M. Babur, L. Mamnani, N. Burrows, B.A. Telfer, F. Carta, J.-Y. Winum, A. Scozzafava, C.T. Supuran, K.J. Williams, Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts, J. Med. Chem. 55 (2012) 5591–5600. doi:10.1021/jm300529u.
[55] E. Bertol, A. Argo, P. Procaccianti, F. Vaiano, M.G. Di Milia, S. Furlanetto, F. Mari, Detection of gamma-hydroxybutyrate in hair: validation of GC-MS and LC-MS/MS methods and application to a real case, J Pharm Biomed Anal. 70 (2012) 518–522. doi:10.1016/j.jpba.2012.07.009.
[56] E. Bertol, F. Mari, G. Orzalesi, I. Volpato, Combustion products from various kinds of fibers: Toxicological hazards from smoke exposure, Forensic Science International. 22 (1983) 111–116. doi:10.1016/0379-0738(83)90002-6.
[57] A.G.W. Leslie, H.R. Powell, Processing diffraction data with mosflm, in: R.J. Read, J.L. Sussman (Eds.), Evolving Methods for Macromolecular Crystallography, Springer Netherlands, 2007: pp. 41–51.
[58] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of Macromolecular Structures by the Maximum-Likelihood Method, Acta Cryst D. 53 (1997) 240–255. doi:10.1107/S0907444996012255.
[59] A.A. Lebedev, P. Young, M.N. Isupov, O.V. Moroz, A.A. Vagin, G.N. Murshudov, JLigand: a graphical tool for the CCP4 template-restraint library, Acta Crystallogr. D Biol. Crystallogr. 68 (2012) 431–440. doi:10.1107/S090744491200251X.
[60] P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, Features and development of Coot, Acta Crystallogr. D Biol. Crystallogr. 66 (2010) 486–501. doi:10.1107/S0907444910007493.
[61] E.M. Westbrook, Crystal-density measurements, in: M.G. Rossmann, E. Arnold (Eds.), International Tables for Crystallography Volume F: Crystallography Ofbiological Macromolecules, Springer Netherlands, Dordrecht, 2001: pp. 117–123. doi:10.1107/97809553602060000664.
[62] S.C. Lovell, I.W. Davis, W.B. Arendall, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson, D.C. Richardson, Structure validation by Calpha geometry: phi,psi and Cbeta deviation, Proteins. 50 (2003) 437–450. doi:10.1002/prot.10286.
[63] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis, J Comput Chem. 25 (2004) 1605–1612. doi:10.1002/jcc.20084.

Supporting Information

Carbonic Anhydrase Inhibitors based on Sorafenib: Design, Synthesis, Crystallographic Investigation and Effects on Primary Breast Cancer Cells

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Table of Content

Table 1. Summary of Data Collection and Atomic Model Refinement Statistics.[1]

	17	**23**
PDB ID	6I0L	6I0J
Wavelength (Å)	0.966	0.966
Space Group	P 21 21 21	P 21 21 21
Unit cell (a,b,c) (Å)	62.46, 70.45, 121.56	62.45, 71.26, 121
Limiting resolution (Å)	1.40	1.35
Unique reflections	105401 (16390)	118885 (18527)
Rsym (%)	4.5 (215.5)	5,1 (342.8)
Rmeas (%)	5.1 (224.1)	5.7 (336.1)
Redundancy	3.46 (3.39)	3.57 (3.45)
Completeness overall (%)	98.6 (96.1)	99,0 (96.6)
<I/(I)>	11.88 (0.58)	10.86 (0.35)
CC (1/2)	99.9 (30.2)	99.9 (12.3)
Refinement statistics		
Resolution range (Å)	28.6-1.4	28.6-1.35
Unique reflections/ working/free	99944 / 4859	112540 / 5325
Rfactor (%)	20.40	19.68
Rfree(%)	23.70	23.25
No. of protein atoms	4050	4051
No. of water molecules	504	581
No. of heterogen atoms	64	80
r.m.s.d. length(Å)	0.0118	0.0112
r.m.s.d. angles (°)	1.449	1.4543
Ramachandran statistics (%)		
Most favored	97.5	97.5
additionally allowed	2.5	2.5
outlier regions	0	0
Average B factor (Å^2)		
All atoms	29.798	26.494
inhibitors	49.991	26.436

solvent	39.805	37.992

[1] Values in parentheses are for the highest resolution shell.